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Rubinstein-Taybi Syndrome Caused by Submicroscopic Deletions within 16p13.3

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Summary

The Rubinstein-Taybi syndrome (RTS) is a well-defined complex of congenital malformations characterized by facial abnormalities, broad thumbs and big toes, and mental retardation. The breakpoint of two distinct reciprocal translocations occurring in patients with a clinical diagnosis of RTS was located to the same interval on chromosome 16, between the cosmids N2 and RT1, in band 16p13.3. By using two-color fluorescence in situ hybridization, the signal from RT1 was found to be missing from one chromosome 16 in 6 of 24 patients with RTS. The parents of five of these patients did not show a deletion of RT1, indicating a de novo rearrangement. RTS is caused by submicroscopic interstitial deletions within 16p13.3 in approximately 25% of the patients. The detection of microdeletions will allow the objective confirmation of the clinical diagnosis in new patients and provides an excellent tool for the isolation of the gene causally related to the syndrome.

Introduction

The Rubinstein-Taybi syndrome (RTS) is a well-known and well-defined complex of congenital malformations, consisting of peculiar facies, broad thumbs and big toes, and variable mental retardation (Michail et al. 1957; Rubinstein and Taybi 1963; Hennekam et al. 1990). In 1990 Imaizumi described a patient with the syndrome and a t(2;16)(p13.3,p13.3) (Imaizumi and Kuroki 1991). At that time it was neither clear whether there was a causal link between the translocation and the syndrome nor which of the breakpoints might indicate the relevant locus (Hennekam 1991; Imaizumi and Kuroki 1991). Since then, two more patients with RTS and a breakpoint in 16p13 have been found, one with t(7;16)(q34;p13.3) (Tommerup et al. 1991) and one with t(16;20) (H. Kääriäinen, personal communication).

The short arm of chromosome 16 is known to carry the mutation responsible for adult polycystic kidney disease (PKD1) (Reeders et al. 1985). During the intensive search for the PKD1 gene, detailed maps for the short arm of chromosome 16 have been created (Harris et al. 1987; Reeders et al. 1988; Callen et al. 1989, 1990; Breuning et al. 1990*b*; Keith et al. 1990; Ceccherini et al. 1992). While building up the chromosome 16 mapping panel, we have established methods for rapid detection of chromosome 16 rearrangements in leukemia by using fluorescence in situ hybridization (FISH) (Dauwerse et al. 1990). The breakpoints of the inv(16), and the t(16;16) characteristic for acute nonlymphocytic leukemia with eosinophylic granulation (ANLL M4-eo) were thus mapped to the same subregion of the short arm of chromosome 16 (Wessels et al. 1991*a*). The breakpoint of the t(8;16) known to occur in patients with acute monoblastic leukemia with erythrophagocytosis, which is cytogenetically indistinguishable from

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the breakpoint of inv(16) or t(16;16), was mapped for the first time to a distinct subregion of 16p (Wessels et al. 1991*b*).

By using FISH, the rank order of approximately 80 cosmids relative to 17 breakpoints in 16p was determined. When slides from two patients with RTS and a reciprocal translocation involving the short arm of chromosome 16 became available, we were able to easily pinpoint these breakpoints within a few days. Using one of the two cosmids that most closely flanked these breakpoints, we discovered submicroscopic deletions in patients with RTS.

Patients, Material, and Methods

All patients have been seen by one of the authors (R.C.M.H.), in the framework of extensive clinical studies of the RTS (Hennekam et al. 1990).

Probes

pHUR 195 is a plasmid containing an alphoid sequence specific for the centromere of chromosome 16 (Moyzis et al. 1987). Chromosome 16 cosmids were obtained from a library prepared from a mouse hybrid cell line containing chromosome 16 as the only human chromosome (Breuning et al. 1990*a*). The N2 cosmids were isolated by screening a cosmid library with the *Not*I linking clone N2 (D16S138) (Himmelbauer et al. 1991; H. Himmelbauer and A.-M. Frischauf, unpublished method).

Cytogenetics

Metaphase and prometaphase spreads of cultured peripheral blood lymphocytes were obtained using standard procedures. G- and R-banding of the chromosomes was performed according to a method described elsewhere (Breuning et al. 1977).

FISH

Probes were labeled by nick-translation (Langer et al. 1981) in the presence of either biotin-11-dUTP or digoxigenin-11-dUTP and were further purified and precipitated, as described elsewhere (Kievits et al. 1990; Dauwerse et al. 1992). Hybridization and detection of the hybrids was performed according to the method described by Pinkel et al. (1986) and Kievits et al. (1990). Two-color FISH was recently described by Dauwerse et al. (1992). Chromosome painting with a pool of 166 cosmids from chromosome 16 (Breuning et al. 1990*a*) was performed according to the method described by Kievits et al. (1990). Analysis was performed with a Leitz Aristoplan microscope equipped for fluorescence microscopy, and suitable metaphases were photographed on 3M 640 ASA diapositive film.

Results

Cytogenetic Studies

Metaphases and prometaphases containing more than 800 bands for all 24 patients showed no detectable chromosome anomaly, particularly not of band 16p13.3.

In Situ Hybridizations

Metaphase spreads of 14 unrelated patients were first studied with four widely spaced cosmids along the short arm of chromosome 16. The cosmids 36 (D16S79), 41 (D16S82), 26 (D16S125), and 40 (D16S257) were used (fig. 1). No deletions or rearrangements were detected. We then tested 16p cosmids on metaphase spreads of the RTS patient with t(7;16)(q34;p13.3). Cosmid N2 was found to be located the closest—i.e., proximal from the breakpoint—and cosmid RT1 distal (figs. 1 and 2, *top left*). The breakpoint of the RTS patient with t(2;16)(p13;p13) (Imaizumi and Kuroki 1991) maps to exactly the same interval of 16p. These cosmids had already been mapped physically very close, and had been ordered, during a parallel search for the breakpoint of t(8;16)(p11;p13) characteristic of ANLL M5 (Wessels et al. 1991*b;* Dauwerse et al. 1992) (fig. 1).

These cosmids cannot be seen separately on metaphase or on prometaphase chromosomes. Therefore, we hybridized both cosmids simultaneously, using different fluorescent labels for N2 (Texas red) and RT1 (FITC, green). Interphase nuclei as well as metaphase spreads from RTS patients were analyzed. If the two signals overlap, then the color turns to yellow (fig. 2, *top right* and *middle left*). Thus in some interphase nuclei and on the metaphase chromosomes, a yellow spot indicated the combined N2/RT1 signal, while red spots indicated the presence of N2 only. In other interphase nuclei, separate green and red spots could be seen representing the normal chromosome 16. In several patients the green signal of RT1 was not visible adjacent to the red spot of N2 (fig. 2, *middle right*).

Slides from 24 patients with RTS were available. In all cases, at least one spot showing both fluorescent labels was seen. However, in 6 of 24 patients, the signal from cosmid RT1 consistently and reproducibly illuminated only one chromosome, whereas the signal from N2 in the other color could be seen in two copies, indicating a deletion of RT1 in these patients (fig. 2, *top right, middle left,* and *middle right*).

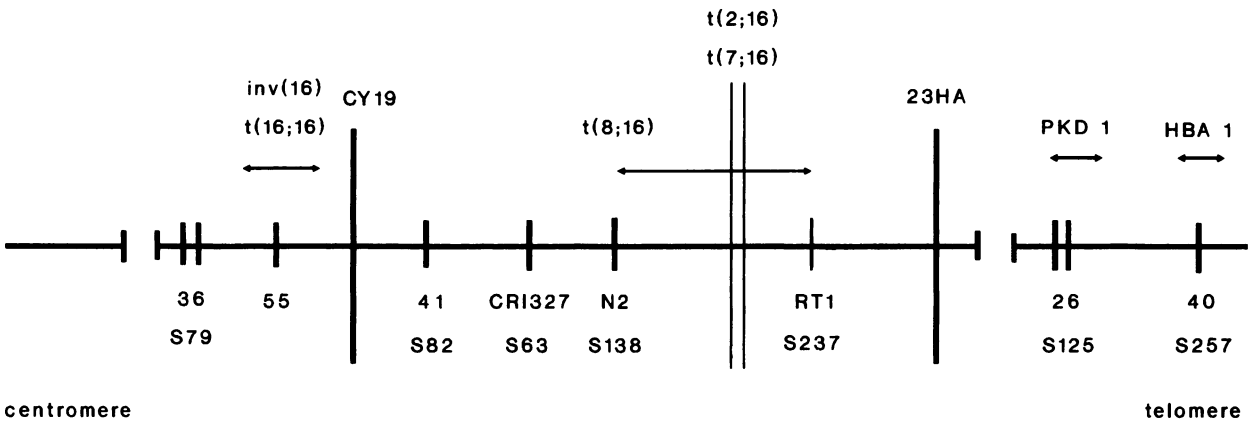

Figure 1 Simplified map of the short arm of chromosome 16, showing the location of the t(2;16) and t(7;16) breakpoints in patients with RTS. The inv(16)/t(16;16) breakpoint is associated with ANLL M4eo, the t(8;16) breakpoint is associated with ANLL M5, and the CY19 and 23HA breakpoints of chromosome 16 are in two somatic cell hybrids (Callen et al. 1990). Cosmids are indicated as follows: 36 (D16S79), 55, 41 (D16S82), CRI-0327 (D16S63), N2 (D16S138), RT1 (D16S237), 26 (D16S125), and 40 (D16S257). PKD 1 = the gene involved in PKD1; and HBA 1 = alpha-globin gene cluster.

Parents of five patients with the interstitial deletion were also available for study. Metaphase spreads were hybridized in one color, with either the N2 or the RT1 cosmid, in separate experiments. In all parents, both N2 and RT1 were present on both chromosomes, indicating that, at least in these five patients, the interstitial deletion had occurred de novo (experiments not shown).

Discussion

The RTS is a well-defined recognizable pattern of human malformation. In many cases the combination of dysmorphic features is characteristic, and the diagnosis can be firmly established. However, since objective means to confirm the clinical diagnosis are lacking, it can be quite difficult to establish or exclude the diagnosis when not all the characteristics of the syndrome are manifest in a patient. Therefore, the finding of a specific chromosomal lesion in 25% of the RTS patients will greatly enhance the reliability of diagnosis in these cases. Furthermore, the prospect of identification of the gene(s) causally related to the syndrome is now realistic, and this will open the way to new, more universal diagnostic procedures.

In the present study, we have concentrated on a well-documented series of patients (Hennekam et al. 1990) with a definitive diagnosis. After pinpointing the breakpoint of two reciprocal translocations involving 16p in RTS patients, we found submicroscopic deletions in 6 of 24 RTS patients with apparently normal chromosomes.

In RTS patients, the mapping of two breakpoints of reciprocal translocations within the same very small interval of chromosome 16 and the discovery of submicroscopic interstitial deletions in approximately 25% of the patients indicate that damage to only one gene is probably critical to the origination of the clinical phenotype. The RT1 cosmid has been found to contain evolutionary conserved sequences, suggesting the presence of some genes (data not shown). The isolation of the gene involved in the origin of the RTS, which is now technically feasible, will shed light on one of the causes of mental retardation and congenital malformation.

With the finding of deletions of chromosome 16 in patients with RTS, this disorder has joined the large group of chromosomal microdeletion syndromes. Such syndromes have been defined on the basis of cytogenetic banding techniques, which showed loss or duplication of entire or partial chromosome bands. The level of resolution of these techniques is up to 1–2 Mb when prometaphase spreads of good quality are available. In the past, it was logical to assume that chromosomal malformation syndromes are the result of the combined effect of aneusomy for several or even many genes. However, the application of gene mapping techniques to these patients reveals that, in fact, very small chromosomal regions are consistently involved in the origin of the syndrome.

The Wolf-Hirschhorn syndrome (chromosome 4), the Langer-Giedeon syndrome (chromosome 8), the WAGR (*W*ilms tumor, *a*niridia, *g*enitourinary malformation, and *r*etardation) syndrome (chromosome 11p),

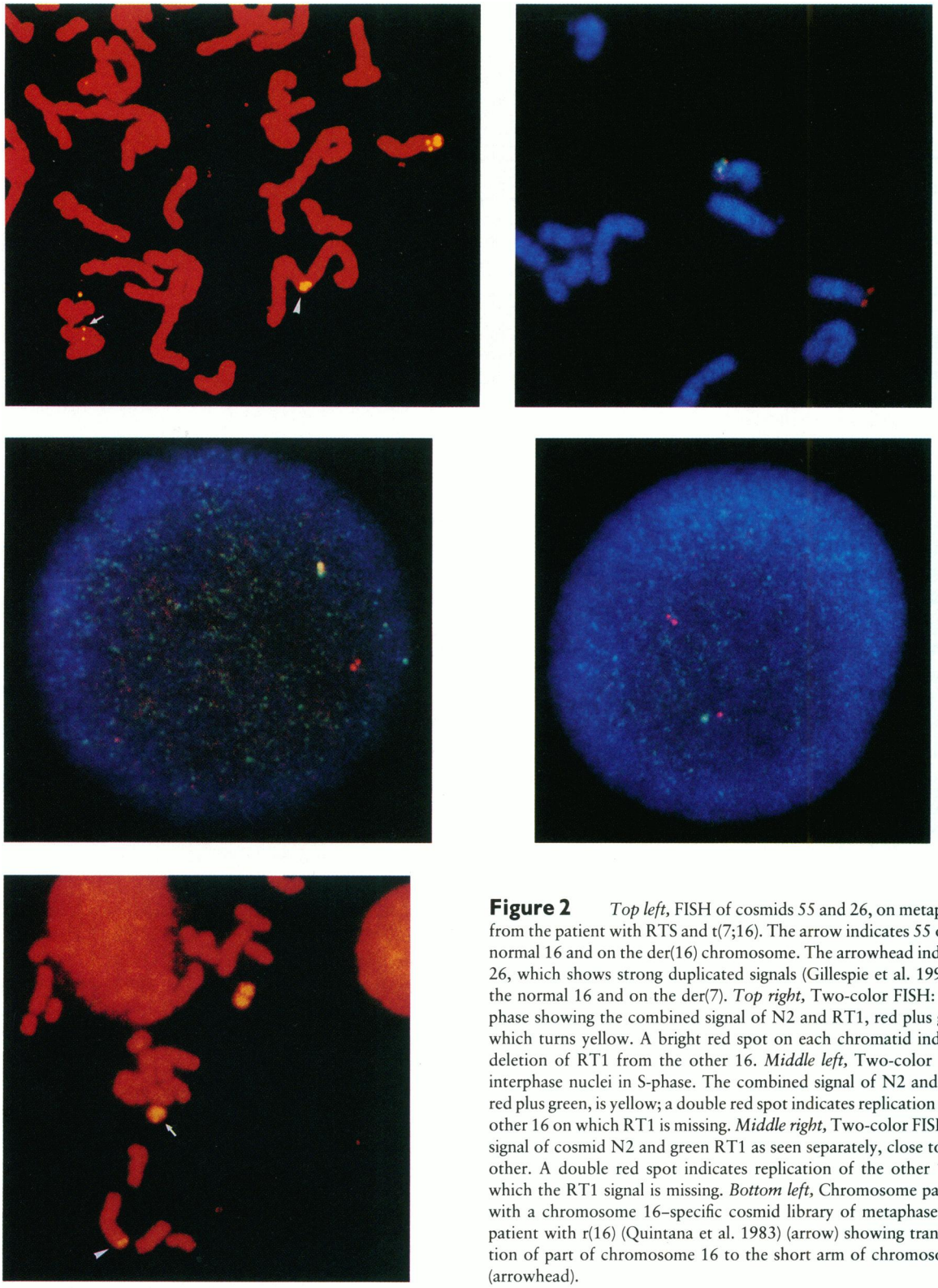

Figure 2 *Top left,* FISH of cosmids 55 and 26, on metaphases from the patient with RTS and t(7;16). The arrow indicates 55 on the normal 16 and on the der(16) chromosome. The arrowhead indicates 26, which shows strong duplicated signals (Gillespie et al. 1990) on the normal 16 and on the der(7). *Top right,* Two-color FISH: metaphase showing the combined signal of N2 and RT1, red plus green, which turns yellow. A bright red spot on each chromatid indicates deletion of RT1 from the other 16. *Middle left,* Two-color FISH: interphase nuclei in S-phase. The combined signal of N2 and RT1, red plus green, is yellow; a double red spot indicates replication of the other 16 on which RT1 is missing. *Middle right,* Two-color FISH: red signal of cosmid N2 and green RT1 as seen separately, close to each other. A double red spot indicates replication of the other 16 on which the RT1 signal is missing. *Bottom left,* Chromosome painting with a chromosome 16–specific cosmid library of metaphases of a patient with r(16) (Quintana et al. 1983) (arrow) showing translocation of part of chromosome 16 to the short arm of chromosome 1 (arrowhead).

the Prader-Willi and Angelman syndromes (chromosome 15q13), the Miller-Dieker syndrome (chromosome 17p13), the Smith-Magenis syndrome (17p11.2), and the Di-George syndrome (chromosome 22) are typical examples. In all these cases, deletions have originally been identified by cytogenetics. Comparison of deletions by using regionally mapped probes shows rapid decreases in the smallest region of overlap in many of these syndromes. When FISH has been used on metaphases from patients with the Miller-Dieker syndrome, submicroscopic deletions resulting from cryptic translocations have been identified by Kuwano et al. (1991). The situation in RTS is different, in the sense that only reciprocal translocations and submicroscopic deletions smaller than 1–2 Mb have been found.

We hypothesize that the key features of RTS are caused by inactivation of a single gene. However, a submicroscopic deletion may also remove other genes, leading to modifications of the phenotype (Hennekam et al. 1993). In any case, the extent of the deletion may well be limited by the presence of flanking genes in chromosome 16p that should be present as a diploid copy so as to ensure survival. In none of the RTS patients did the deletion extend beyond the N2 cosmid proximally or the 26 cosmid distally. This notion is supported by the absence of extensive 16p deletions from the literature (Schinzel 1984; Wilkie et al. 1990). Only one patient with a ring 16 chromosome (Quintana et al. 1983) appeared to have a deletion from D16S79 (cosmid 36) to the telomere (Callen et al. 1989). However, after further scrutiny of this r(16) with FISH, using both single cosmids and painting with a pool of chromosome 16 cosmids, a more complex rearrangement with translocation of the 16p13 band to the short arm of chromosome 1 was shown (fig. 2, *bottom left*). Thus monosomy of only small fragments of 16p seems to be compatible with life.

These results show the great power of FISH when it is combined with adequate diagnosis and mapping information. Further analysis of RTS patients by using single-copy probes from the RT1 cosmid is now under way. In addition, isolation and characterization of cDNAs from this region should give us the RTS gene. As of today, FISH using the RT1 cosmid can reliably identify a submicroscopic deletion in about 25% of the patients and can confirm the clinical diagnosis of RTS. It can be expected that, in analogy with other microdeletion syndromes and single-gene disorders, an additional fraction of the patients may show detectable mutations when new tools and more refined methods are available. The question as to whether the 75% nondeleted patients have a mutation in one and the same gene on chromosome 16 (reflecting the presence or absence of etiologic heterogeneity in the RTS) remains to be answered.

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